MEDICAL STAFF CONFERENCE

Primary Hyperoxaluria

These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California Medical Center, San Francisco. Taken from transcriptions, they are prepared by Drs. Martin J. Cline and Hibbard E. Williams, Assistant Professors of Medicine, under the direction of Dr. Lloyd H. Smith, Jr., Professor of Medicine and Chairman of the Department of Medicine.

DR. SMITH:* Today we shall present a new genetic disease which has been studied extensively in this Medical Center during the past year. The findings in the case of our patient today were presented recently at one of the national research meetings,[5] but have not previously been discussed here. The disease which the patient has is a very rare genetic disorder. It is not of itself of practical importance to a general medical audience because of its rarity. It is presented, however, as a case history of how this type of metabolic disease is recognized and pursued. In this regard we have asked Dr. Hibbard Williams, Assistant Professor of Medicine, to serve as a triple threat man; he is going to present the case summary, and then discuss the disease entity with emphasis on his recent findings concerning the pathogenesis of this disorder.

DR. WILLIAMS:*[1] The patient is a 32-year-old Negro male hotel worker. He was well throughout his life until 1958 when flank pain and dysuria developed and he then was admitted to a hospital, where bilateral nephrolithiasis was found. He was placed on a low calcium diet and felt well until about a year later, when he had a second episode of renal colic. Following the episode he was free of symptoms except for occasional cloudy urine until, in 1965, he was admitted to the Veterans Administration Hospital in San Francisco with severe left renal colic. An intravenous pyelogram demonstrated a stone blocking the left ureter. Left ureterostomy was performed and several small stones were removed. These stones were analyzed and found to contain calcium oxalate. At this time a urinary oxalate determination performed in our laboratory showed the level to be elevated at 140 mg in 24 hours. Since that time the patient has felt well and has continued working. He has had two minor episodes of renal colic with spontaneous passage of small stones on each occasion. There was no family history of renal colic, nephrolithiasis or diabetes mellitus. Results of physical examination were relatively unremarkable. The blood pressure was 110/65 mm of mercury. Abdominal examination was within normal limits; the kidneys were not palpable and there was no costovertebral angle tenderness.

Laboratory data: Hematocrit 40 percent. Leukocyte count was within normal limits, as was the differential smear. Urinalysis demonstrated a trace of protein, pH6.0, specific gravity 1.013. On microscopic examination of the sediment there were numerous calcium oxalate crystals and a few red blood cells. Urine culture on several occasions has been negative. Serum calcium, phosphorus, alkaline phosphatase, creatinine and uric acid were all within normal limits. The 24-hour urine calcium was within normal limits, as was creatinine clearance. Urinary cystine was negative, high voltage electrophoresis of both urine and blood

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*[1] Hibbard E. Williams, M.D., Assistant Professor of Medicine.

demonstrated a normal amino acid pattern. Urinary oxalate was 140 mg in 24 hours, (normal 10 to 55), 24-hour urinary glycolate was 32 mg in 24 hours (normal, 15 to 60).

During the past year the patient has been treated with a high fluid and high phosphate intake, and pyridoxine 150 mg per day. Unfortunately he was unable to come to the hospital today. Dr. Youker, will you present the x-ray findings?

DR. YOUKER:*[2] The chest film was within normal limits. The abdominal film shows numerous small calcifications in the right kidney, and an additional radioopacity in the left kidney. These radioopacities are small and extremely dense, suggesting that they are composed of calcium oxalate, the most radioopaque of the materials contributing to renal calculi. In the lower pole of the right kidney these calculi are faceted, which indicates that they are all in the same dilated calyx.

DR. WILLIAMS: In 1792, Alexander Philip Wilson commented in his book, "An Inquiry into the Remote Cause of Urinary Gravel":

> "Here a very important question occurs: does the body by its own powers generate an acid capable of precipitating from the urine, or is such an acid always derived from a cessant diet? Several of the above experiments seem to show that this acid is constantly generated in the body independent of all acids derived from the alimentary canal, and from circumstances already taken notice of, it may pass in great quantity by the kidney."

Today, some 175 years later, I would like to describe two diseases that are caused by the accumulation of organic acids, which are "generated in excess in the urine and passed in great quantity by the kidney."

The subject is primary hyperoxaluria.[2] Although my discussion will concern only a small number of patients and a very rare disease or diseases, it may have broader implications. Approximately two-thirds of all kidney stones contain calcium oxalate. Study of patients similar to the patient presented today may lead to a greater understanding of factors controlling oxalate excretion and thereby benefit patients with oxalate stones of any cause.

To begin, I would like to review the syndrome of primary hyperoxaluria, as it is now known. Primary hyperoxaluria is, first of all, a rather rare genetic disorder. There are some 75 recorded cases in the world literature. It is associated with onset of symptoms early in life, usually between the ages of one and six, and with early death, usually before the age of 20. The major clinical manifestation is recurrent calcium oxalate nephrolithiasis. This is associated with the familiar symptoms of flank pain, dysuria and recurrent urinary tract infection. Nephrocalcinosis also occurs, as does progressive renal failure, which usually leads to death at an early age. The term *oxalosis* refers to the extrarenal deposits of calcium oxalate. These are not specific for primary hyperoxaluria, for they have been found in conditions other than hyperoxaluria. In patients who have died with hyperoxaluria there does seem to be a predilection for calcium oxalate deposition in the myocardium, in the genitourinary system and in the bone marrow. These are rarely of any clinical significance. Two cases have been described with complete heart block, presumably due to calcium oxalate deposition in the conduction system of the heart. The disease is inherited presumably by an autosomal recessive mode of inheritance, but at the present time no definitive heterozygote defect can be demonstrated.

The diagnosis of primary hyperoxaluria is based on the demonstration of an elevated urinary oxalate level. Normal excretion is approximately 10 to 55 mg in 24 hours. In patients with primary hyperoxaluria the excretion of urinary oxalate varies between 150 and 400 mg in 24 hours — approximately three to ten times normal. Besides oxalate, two other organic acids, glycolic acid and glyoxylic acid, are excreted in excess in patients with this syndrome.[3]

Chart 1 is an outline of the synthetic pathways of oxalic acid. Oxalate itself represents a metabolic cul-de-sac. It is not metabolized. All radio-

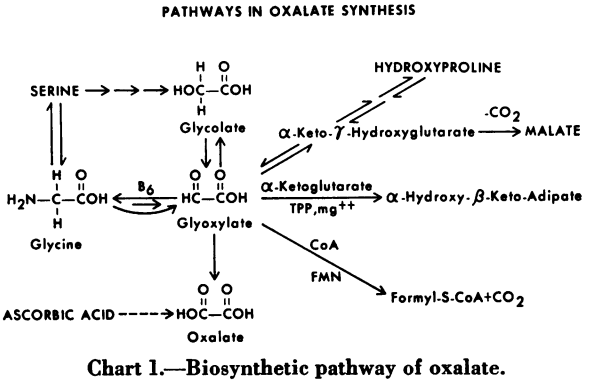


Chart 1.—Biosynthetic pathway of oxalate.

*[2] James E. Youker, M.D., Associate Professor of Radiology.

active oxalate administered to a normal person is recovered unchanged in the urine. Previous studies in patients with hyperoxaluria have demonstrated that oxalate is overproduced from its immediate precursor, glyoxylate. As was previously mentioned, both glyoxylate and glycolate are increased in the urine in patients with hyperoxaluria. Previous studies[1] from Dr. Smith's laboratory have demonstrated a defect in the metabolism of glyoxylate in these patients, which accounts for the increased synthesis and excretion of oxalate.

Glyoxylate has three major pathways of metabolism: transamination to glycine, reduction to glycolate, and reaction with alpha-ketoglutarate to form the compound alpha-hydroxy-beta-keto-adipate. Since glycolate is increased in the urine in patients with hyperoxaluria, the reduction of glyoxylate to glycolate is unimpaired in patients with hyperoxaluria. In the past year we have studied the transamination of glyoxylate to glycine in tissue from four patients with hyperoxaluria, and have found this reaction to be normal.[8] Therefore, we focused our attention on the remaining pathway of metabolism of glyoxalate, controlled by a specific carboligase enzyme. In five patients with primary hyperoxaluria, this enzyme was diminished in cytoplasmic fractions from liver, spleen and kidney.[4] Therefore this enzymatic block in the conversion of glyoxylate to alpha-hydroxy-beta-keto-adipate represents the metabolic error in hyperoxaluria, leading to accumulation of glyoxylate behind the block, and subsequent increased synthesis and excretion of oxalate.

In summary, primary hyperoxaluria is a rare genetic disorder, characterized by recurrent calcium oxalate nephrolithiasis, leading to chronic renal failure and death at an early age. Increased amounts of oxalic, glyoxylic and glycolic acids are found in the urine. Previous studies have demonstrated a defect in metabolism of glyoxylate in this syndrome, and the enzyme defect appears to be a deficiency of a specific carboligase important in glyoxylate metabolism.

I would like to return to the case presented today. The patient was referred to us because of calcium oxalate nephrolithiasis, and, as noted, the urinary oxalate excretion was found to be elevated at 140 mg in 24 hours. This was high enough to be commensurate with a diagnosis of primary hyperoxaluria.

It is customary in this laboratory when an elevated oxalate is found, to carry out a urinary glycolate determination to confirm the diagnosis of primary hyperoxaluria. In the present case two interesting things occurred when this measurement was attempted.

First of all, the absolute level of glycolate was normal, a finding which separated this patient from other patients with primary hyperoxaluria. The second finding is related to the method of measuring urinary glycolate. In order to measure glycolate, a relatively specific color test is used on certain fractions of the urine. When this was performed on urine from today's patient an abnormal color was noted. We therefore began to investigate the reason for this abnormal color, and the first approach to this was through paper chromatography.

Figure 1 represents a paper chromatogram of some urine fractions after column chromatography. On the left is a urine sample from a normal person. The lower spot is unidentified, the upper spot is glycolic acid. The middle urine sample is from a patient with primary hyperoxaluria. The sample on the far right represents the urine of the patient in the present case. A faint glycolic acid spot is discernable, and adjacent to it and overlapping it somewhat is a rather large spot which obviously explained the problems we had had in performing the glycolic acid color test. Here, in fact, was a new compound in the urine which was not found in the urine of other patients with primary hyperoxaluria, nor was it found in the urine of normal subjects, of patients with idiopathic hypercalciuria, or of patients with renal tubular acidosis. At about this same time, we were fortunate to obtain from England urine specimens from two children whom Dr. Smith had studied four years previously. These were siblings, aged eight and ten, who had had nephrolithiasis since infancy. Both had hyperoxaluria but normal levels of glycolate. Paper chromatography of urine fractions from both patients yielded the same abnormal spot as just described in the urine of the patient in today's case.

Our attention was focused on the identification of this abnormal compound. By a variety of techniques including column and paper chromatography, specific color reaction, substrate specificity and derivative analysis, this compound was identified as glyceric acid. Through the kindness of Dr. John Craig of the Department of Pharmaceutical Chemistry the compound was found to exist en-

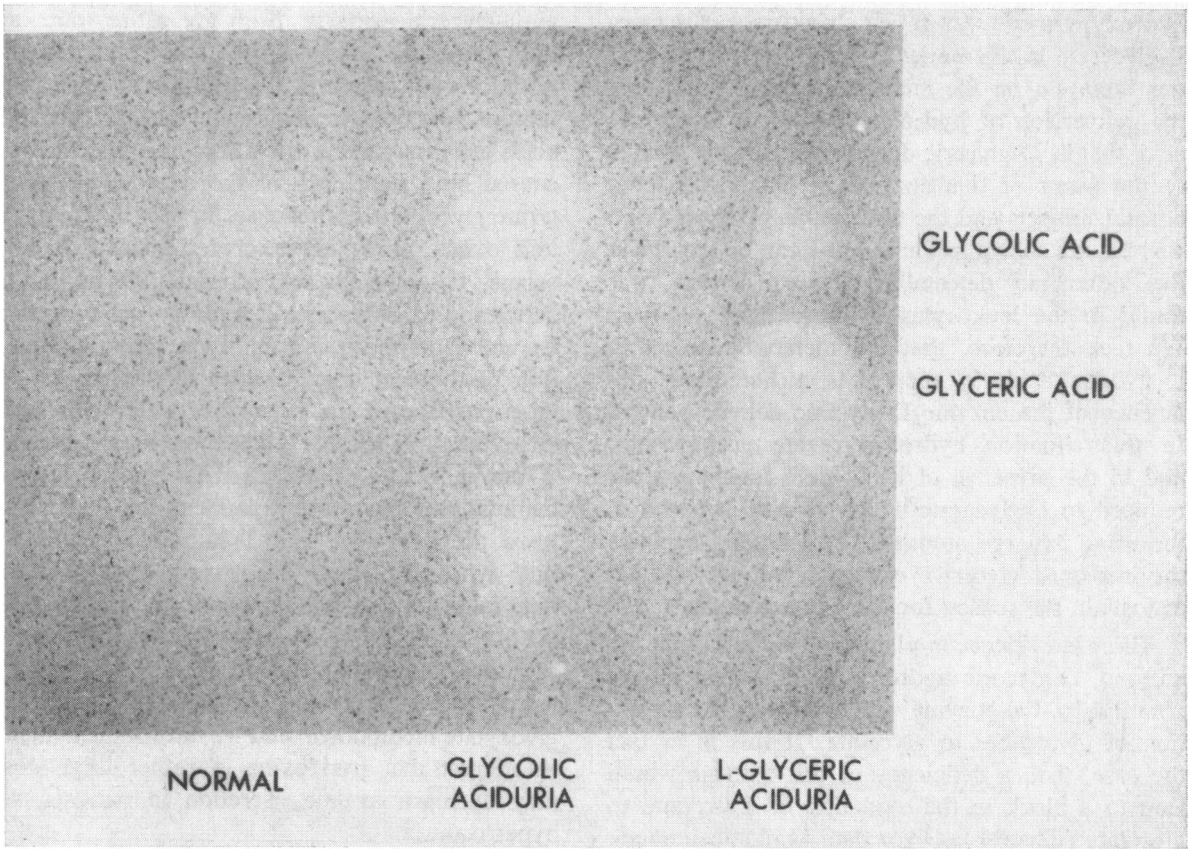
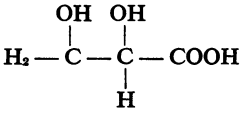


Figure 1.—Paper chromatogram of urine fractions from a normal subject and patients with primary hyperoxaluria. (See text.)

tirely as the L-stereoisomer. The chemical structure of this compound is shown below:



By an isotope dilution technique it was possible to measure the total amount of glyceric acid being excreted.[7] In the three patients with this new syndrome, glyceric acid excretion varied between 196 and 638 mg in 24 hours.

We were left with two rather major questions at this point. First, why was glyceric acid present in the urine, and second what was the association with primary hyperoxaluria? The metabolic pathways involving glyceric acid are shown in Chart 2. D-glyceric acid is an intermediate in the pathway of serine metabolism. Serine may be transaminated to the compound, hydroxy-pyruvate, which is reduced to D-glycerate, which in turn is converted to 2-phosphoglycerate. D-glycerate, then is an intermediate metabolite on the gluconeogenic pathway of serine. Hydroxypyruvate can also be reduced to L-glyceric acid in the presence of lactic dehydrogenase. Since only L-glyceric acid was found in urine, a metabolic block in the conversion of hydroxypyruvate to D-glycerate could explain the increased synthesis and excretion of L-glycerate in these patients.

In order to test this hypothesis, C^{14}-labeled hydroxypyruvate was administered to the patient in our hospital, and over 15 percent of the radioactively labeled material was recovered in urinary glycerate, a finding which demonstrated that

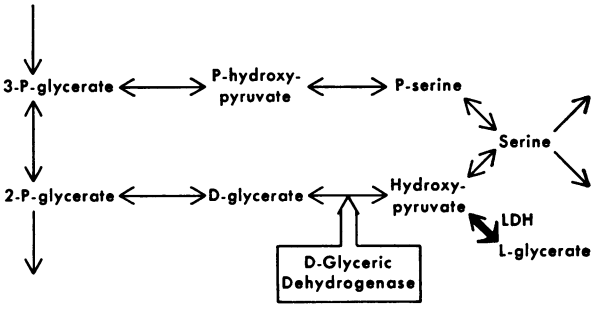


Chart 2.—Metabolic pathways involving glyceric acid. LDH = lactic dehydrogenase.

hydroxypyruvate was in fact a precursor of urinary L-glycerate in this patient.[6] We therefore focused our attention on the enzyme reaction controlling the conversion of hydroxypyruvate to D-glyceric acid, that is, D-glyceric dehydrogenase. The results of the assay of this enzyme in leukocytes from normal subjects and the three patients whose urine we had, are shown in Table 1. As can be seen from this table, no detectable enzyme activity was found in the leukocytes of these three patients. We feel, therefore, that the metabolic defect in L-glyceric aciduria represents a hereditary deficiency of the enzyme D-glyceric dehydrogenase. In this situation hydroxypyruvate accumulates, and in the presence of lactic dehydrogenase it is reduced to L-glycerate which is then excreted in the urine in large amounts. This finding explains the increased glycerate excretion but we had yet to explain the reason for the hyperoxaluria.

There is evidence in plants and bacteria that the enzyme, D-glyceric hydrogenase is similar, if not identical to, the enzyme which controls the reduction of glyoxylate to glycolate. If this is in fact the case, then a deficiency of this enzyme would lead to a block in the oxidation of glyoxylate to glycolate (Chart 1). Glyoxylate would accumulate and this would account for the increased synthesis and excretion of oxalate in the urine of these patients. This is currently our working hypothesis but we have no definitive proof that this is indeed the case.

In summary, L-glyceric aciduria is a rare genetic disease, associated with recurrent calcium oxalate nephrolithiasis. Urinary oxalate is increased but glycolate is normal. L-glyceric acid is found in large amounts in the urine. The enzyme defect represents a deficiency of D-glyceric dehydrogenase.

The differentiation of primary hyperoxaluria from this new syndrome is shown in Table 2. Type 1 is referred to as glycolic aciduria, and type 2 as L-glyceric aciduria. Both are rather rare, and both are characterized by the same clinical manifestation — recurrent calcium oxalate nephrolithiasis. In type 1, oxalic, glycolic and glyoxylic acids are increased in the urine, and the disease is caused by a deficiency of the enzyme 2-oxo-glutarate glyoxylate carboligase. In type 2, L-glyceric and oxalic acids are excreted in the urine in excess, but glycolic acid excretion is normal. A deficiency of D-glyceric dehydrogenase represents the metabolic basis for this type. The differentiation of the two types can be made only by the determination of the urinary organic acids or by demonstration of the specific enzyme deficiency.

We know of no ill effects from glyceric acid accumulation. Therefore treatment is directed toward the hyperoxaluria. Treatment is similar in both types of primary hyperoxaluria — increased fluid intake to decrease the concentration of solutes in the urine, high phosphate intake to inhibit crystal formation, and pyridoxine in doses of 150 mg per day. Pyridoxine is a co-factor important in glyoxylate metabolism, and we have some evidence to suggest that pyridoxine in rather large doses may decrease oxalate excretion in patients with hyperoxaluria.

In conclusion, I would like to relate to you a comment which was traced to a response of the oracle at Delphi, which was given to Polycrates as the best means of finding a treasure buried by Xerxes' general, Mardonius, on the field at Plateia. The oracle reputedly said, "Leave no stone unturned."

DR. SMITH: I don't know how to respond to that last lithic pun. There is time for questions or comments concerning this rather rare disease and its investigation.

DR. FUDENBERG:*[3] How many children are in the family and what is the sex distribution? I gather all important data have been obtained from males.

TABLE 1.—*Leukocyte D-glyceric dehydrogenase activity*

Subject	*Enzyme activity in mμ moles/mg prot./hr.*
Normal Range	12.2-24.3
Mean	18.2
Patients with L-Glyceric aciduria	
Subject patient	0
*10-year-old child	0
* 8-year-old child	0

*Two English children, mentioned in text, from whom urine specimens were available.

TABLE 2.—*Differentiation of two types of primary hyperoxaluria*

Type I	(Glycolic aciduria)
	Clinically recurrent nephrolithiasis
	Urinary organic acids—oxalic and glycolic
	Enzyme defect.—2-oxo-glutarate: glyoxylate carboligase
Type II	(L-glyceric aciduria)
	Clinically recurrent nephrolithiasis
	Urinary organic acids—oxalic and glyceric
	Enzyme defect—D-glyceric dehydrogenase

*[3]H. Hugh Fudenberg, M.D., Professor of Medicine.

DR. WILLIAMS: No, I am sorry I did not specify. There are two sons and one daughter in the family, all affected.

DR. FUDENBERG: Do you plan to do studies on relatives?

DR. WILLIAMS: Yes, we have blood specimens from the parents of the two English children. The activity of the enzyme is low in the mother's leukocytes; in the father's leukocytes it is low normal but still within the normal range.

Editor's follow-up: Shortly after presentation of the case at the staff conference, the patient was admitted to hospital because of acute diabetic ketoacidosis. He responded promptly to insulin and is now being treated with daily insulin therapy. He has had no further episodes of renal colic. The relationship of the diabetes mellitus to his other hereditary disease is as yet unknown.

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